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J. Parasitol., 88(6), 2002, pp. 1187–1199

ASHWORTHIUS PATRICIAPILITTAE N. SP. (TRICHOSTRONGYLOIDEA: HAEMONCHINAE), AN ABOMASAL NEMATODE IN *ODOCOILEUS VIRGINIANUS* FROM COSTA RICA, AND A NEW RECORD FOR SPECIES OF THE GENUS IN THE WESTERN HEMISPHERE

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ABSTRACT: A species of *Ashworthius* is reported for the first time in the Western Hemisphere, and *A. patriciapilittae* n. sp. is described on the basis of specimens in white-tailed deer *Odocoileus virginianus* from Costa Rica. Among 8 known species, *A. patriciapilittae* is morphologically similar to *A. tuyenquangi* in red muntjac *Muntjacus muntjak* from northern Vietnam. The synlophe in *A. patriciapilittae* is composed of 26 ridges in the cervical zone and is continuous to the caudal extremity in males and females. Males are characterized by a complex dorsal ray and narrow trifurcate spicules (351–356 μm long) lacking an ''eyelet,'' with dissimilar ventral and dorsal processes; the gubernaculum is 45–48% of the spicule length. Females have a prominent linguiform flap at the vulva and large eggs (108–142 μm long). The presence of *A. patriciapilittae* in Costa Rica is examined in the context of competing hypotheses for cospeciation or contemporary host-switching in cervids; either *A. patriciapilittae* is a component of an endemic Central and South American fauna that has diversified through coevolution of *Ashworthius* and cervid hosts or it has been introduced. Among haemonchines in the Western Hemisphere, specimens of *A. patriciapilittae* may be confused with 3 species of *Haemonchus*, including *H. contortus*, *H. placei*, and *H. similis*, that occur in both domestic and wild ruminants. Discovery of *A. patriciapilittae* emphasizes the continued need for survey and inventory to define the structure and distribution of parasite faunas in wild and domestic ruminants from the Nearctic and Neotropical regions.

Ashworthius Le Roux, 1930, was established for trichostrongyle nematodes, morphologically similar to species of *Haemonchus* Cobb, 1898, occurring in the abomasum of wild ruminants. Originally distinguished from species of *Haemonchus* by a symmetrical dorsal lobe of the bursa and absence of a gubernaculum (Le Roux, 1930), the generic diagnosis was later modified to include characters of the synlophe (Durette-Desset and Chabaud, 1977, 1981; Gibbons and Khalil, 1982; Durette-Desset, 1983). Diagnostic characters for *Ashworthius* spp. apparently require reevaluation, however, because the gubernaculum may be present or absent (e.g., Pike, 1969; Chauhan et al., 1972) and the synlophe may be variable in extent and similar with respect to some *Haemonchus* spp. (Lichtenfels and Pilitt, 2000).

There are currently 7 species of *Ashworthius* that occur primarily in species of Bovidae or Cervidae from sub-Saharan Africa and Eurasia, including the Indian subcontinent (Pike, 1969); an exception is *A. leporis* Yen, 1961, in a leporid *Lepus mandshuricus* Radde from China (Yen, 1961). In bovid hosts, 4 species have been described among sylvatic representatives of the Bovinae and rarely Antilopinae. The type species *A. pattoni* Le Roux, 1930, is a parasite in bushbuck *Tragelaphus scriptus sylvaticus* Sparrman, from Natal, South Africa (Le Roux, 1930). *Ashworthius martinagliai* Ortlepp, 1935, was described in nilgai *Boselaphus tragocamelus* Pallas, imported from India to the Johannesburg Zoological Gardens (Ortlepp, 1935). *Ashworthius lerouxi* Diaouré, 1964, was described in dwarf forest buffalo *Syncerus caffer nanus* Boddaert from the Congo and is also known in *S. caffer caffer* Sparrman from Zambia (Diaouré, 1964; Pike, 1969). Most recently, Chauhan et al. (1972) described *A. perilli* Chauhan, Pande, and Singh, 1972, in nilgai and blackbuck *Antilope cervicapra* Pallas from India. There is no record of any species of *Ashworthius* in domestic bovids.

In cervids, 2 species have been described primarily among the Cervinae; contemporary host-switches are evident in some species of Odocoileinae and Bovidae. *Ashworthius sidemi* Schulz, 1933, was described in sika deer *Cervus nippon hortulorum* Swinhoe, from the Russian Far East near Vladivostok (Schulz, 1933); it also has been recognized in red deer, *C. elaphus sibiricus* Severtzov (reported as *C. elaphus asiaticus* Lydekker), from south-central Asia and in sambar, *C. unicolor* Kerr, from northern Vietnam (Dróżdż, 1973). In addition, *A. sidemi* includes the synonym *A. gagarini* Kostyaev, 1969, described in *C. elaphus sibiricus* from the Altai Mountains, Kazakhstan (Dróżdż et al., 1998; Ferté et al., 2000). Although *A. sidemi* is considered typical in Asiatic cervids, this species has been widely introduced with the translocation of sika deer and is a known colonizer of *C. elaphus sibiricus*, *C. elaphus elaphus* Linnaeus, *Capreolus capreolus* Linnaeus, *Alces alces* Linnaeus, *Ovis musimon* Pallas, and *Bison bonasus* (Linnaeus) in the central to western Palearctic (Ferté and Durette-Desset, 1989; Dróżdż et al., 1998; Ferté et al., 2000). In contrast to *A. sidemi*, with a relatively broad host and geographic distribution, *A. tuyenquangi* Dróżdż, 1970, was described in red muntjac *Muntjacus muntjak* Zimmermann from northern Vietnam (Dróżdż, 1970).

Carreno et al. (2001) reported an apparently undescribed species of *Ashworthius* in white-tailed deer *Odocoileus virginianus* (Zimmermann) from Costa Rica. There was no prior record for species of *Ashworthius* among ruminants from the Western Hemisphere, although these nematodes were recognized among potential pathogens that could be introduced with the translocation of exotic cervids or bovids from Africa and the Palearctic (Hoberg et al., 2001). In addition, the report by Carreno et al. (2001) constituted the first record for an apparently natural infection of these nematodes among any representative of the tribe Odocoileini. In the current study, we describe this previously unrecognized species of *Ashworthius* and explore hypotheses for the distribution of these nematodes in the Nearctic and Neotropical regions.

Received 4 February 2002; revised 6 June 2002; accepted 6 June 2002.

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MATERIALS AND METHODS

Specimens of an undescribed species of *Ashworthius* were found in the abomasum of an adult female white-tailed deer *O. virginianus truei* Merriam collected within the Area de Conservación Guanacaste, Costa Rica (10°57′N, 85°48′W), in early June 1999 (Carreno et al., 2001). Permits for the collection and other details of the necropsy are outlined in Carreno et al. (2001). In the absence of a centralized infrastructure for parasitological collections in Costa Rica, the holotype, allotype, and paratypes are deposited in the U.S. National Parasite Collection (USNPC) at the Parasite Biology, Epidemiology, and Systematics Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland (www.anri.barc.usda.gov.pbesl).

Nematodes were prepared as temporary whole mounts, cleared in phenol–alcohol, and examined using interference contrast microscopy. Characters studied have been previously outlined in detail (Hoberg et al., 1993). The synlophe (Durette-Desset, 1983; Lichtenfels et al., 1988) was studied in whole mounts; cross sections of 1 male specimen were prepared by hand. Terminology for the cervical synlophe among haemonchines follows concepts developed by Lichtenfels et al. (1994), Lichtenfels and Pilitt (2000), and Lichtenfels et al. (2002). Bursal ray patterns and numbering of the bursal papillae are consistent with the findings of Chabaud et al. (1970) and Durette-Desset and Chabaud (1981). Measurements are in micrometers unless indicated otherwise and are presented as the range followed by the mean ± 1 standard deviation in parentheses; description and measurements are from 3 males and 3 females, except where specified otherwise (in cases where n is provided). Taxonomy for the trichostrongyles and the Haemonchinae, as applied in the current study, is consistent with those in Durette-Desset (1983), Hoberg and Lichtenfels (1994), and Lichtenfels and Pilitt (2000).

Comparative specimens are (1) *A. sidemi* Schulz, 1933, USNPC 89169, voucher specimens in *C. elaphus sibiricus* from the Altai Mountains, Kazakhstan, (2) *A. lerouxi* Diaouré, 1964, USNPC 66647, voucher specimens in *S. caffer nanus* from Uganda, (3) *A. tuyenquangi* Dróżdż, 1970, in *M. muntjak* from Vietnam; some syntype specimens held at Polish Academy of Sciences were transferred to USNPC 91946.

MORPHOLOGICAL DESCRIPTION

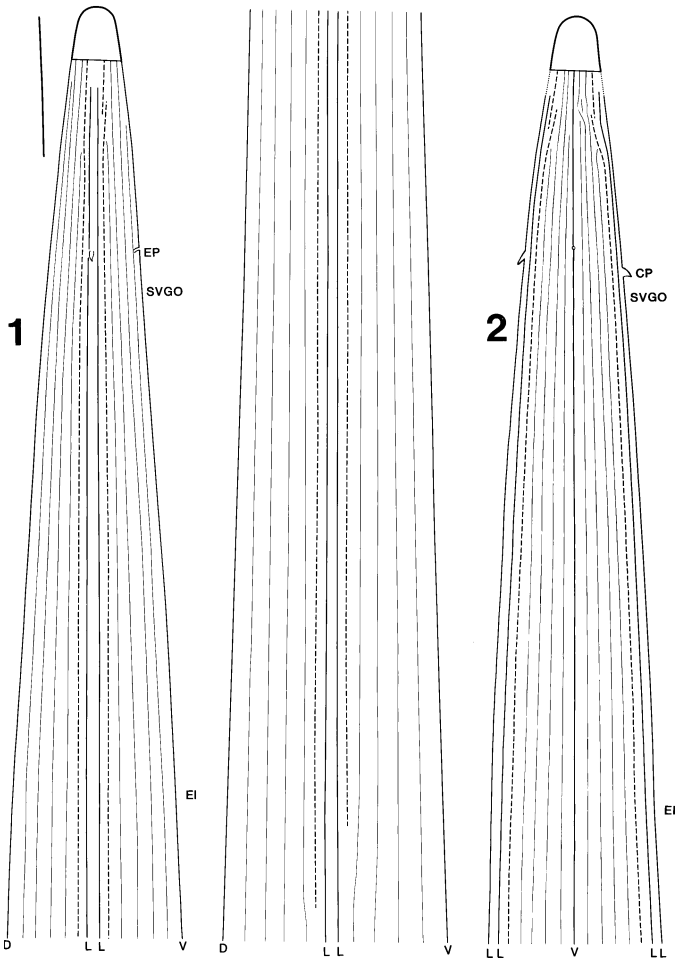
Ashworthius patriciapilittae n. sp.

(Figs. 1–32)

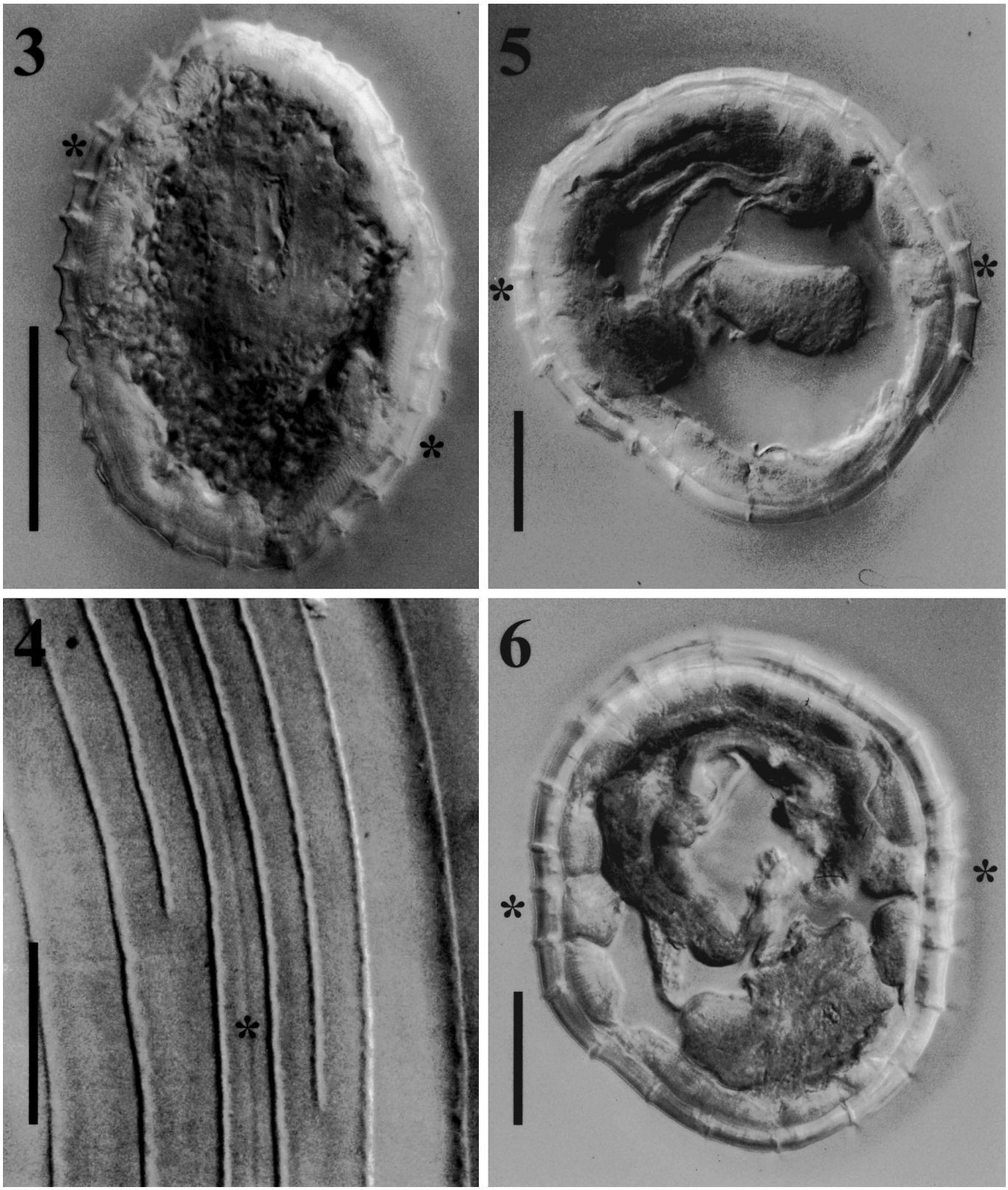
General description: Trichostrongylidae, Haemonchinae, uncoiled. Cephalic expansion present. Esophageal tooth lancetlike, dorsally recurved, triangular in ventrodorsal view, extending into buccal capsule; orifice of dorsal esophageal gland in base of tooth. Cuticle with fine transverse annulations. Synlophe conspicuous, extending from base of cephalic expansion throughout body length to near caudal extremity in males and females. Esophagus attaining maximum width at base, valve absent at esophageal–intestinal junction (EIJ). Subventral esophageal gland orifices (SVGO) posterior to prominent triangular cervical papillae (CP) and excretory pore.

Synlophe: Bilaterally and dorsoventrally symmetrical synlophe identical in males and females; ridges perpendicular to body wall. Cervical zone with 18–20 ridges originating at the base of the cephalic expansion; increasing to 26 ridges at the level of cervical papillae. Ventral and dorsal fields, each with 7 ridges; paired subdorsal and subventral ridges absent. Lateral fields, each with 6 ridges as defined by relationship to sublateral cords: lateral-most ridges paired, continuous to caudal extremity; inner sublateral ridges (number 1 sublaterals) paired, originating at cephalic expansion, terminating at 2,165–3,205 from the cephalic extremity or is 15–23% the body length from the anterior in males and 2,480–3,572 or 13–22% of the body length in females; median sublateral ridges (number 2 sublaterals) absent; outer sublateral ridges (number 3) originating anterior to CP, extending from cervical zone to near tail in right and left fields. Ridges in lateral fields more robust and larger than those in dorsal and ventral fields. Number of ridges decreasing from 26 to 22 near the end of the first quarter of the body: synlophe in midbody, and third quarter composed of 22 ridges in males and females, with 4 ridges in each lateral field and 7 ridges in each dorsal and ventral field. Through posterior quarter, anterior to spicules in males, ridges in dorsal and ventral fields terminate, leaving 4 ridges extending to prebursal papillae (PBP) in each lateral field; in 1 male, 2 dorsal-most and 2 ventral-most ridges end 950–1,000 anterior to PBP or 93% of the body length from anterior. In females, synlophe terminates slightly posterior to anus.

Male: Body 12,925–14,825 (13,950 ± 958.8) long. Esophagus 1,135–1,262 (1,207 ± 65.3) long, 96–117 (103 ± 12.1) in maximum width at base; 8.5–8.7% total body length; body width at base of esophagus 143–198 (170 ± 27.5). Nerve ring



FIGURES 1–2. *Ashworthius patriciapilittae* n. sp., synlophe in anterior of male and female specimens. Bar = 100 μm. D = dorsal; CP = cervical papillae; EI = esophageal–intestinal junction; EP = excretory pore; L = lateral; SVGO = subventral gland orifice. **1.** Cervical synlophe and continuation of the postcervical synlophe posterior to the EI in lateral view showing paired lateral-most ridges (L), and number 1 sublateral ridges (dashed lines) terminating in anterior 1/4 of body. **2.** Cervical synlophe in ventral view, showing single ventral ridge and number 1 sublateral ridges (dashed lines).



FIGURES 3–6. *Ashworthius patriciapilittae* n. sp., photomicrographs showing characteristics of the synlophe (* marks lateral). Bars = 50 μm. **3.** Cross section of male, near level of EI, showing 26 ridges. **4.** Lateral synlophe of male showing posterior ends of number 1 sublateral ridges adjacent to lateral-most ridges. **5.** Cross section of male near midbody showing 22 ridges. **6.** Cross section of male near 3/4 of body length showing 22 ridges.

280–315 (301 ± 18.5), excretory pore 335–400 (369 ± 32.6), CP 360–422 (393 ± 31.3), and SVGO 450–498 (473 ± 24.10) from cephalic extremity. Copulatory bursa large, symmetrical, strongly bilobed, 514–588 (555 ± 37.6) long from minuscule PBP; body width anterior to bursa at PBP 224–270 (251 ± 24.1). Pattern of bursal rays 2-1-2; ventral and lateral rays robust; tips of rays 2 and 3 slightly convergent, attaining bursal margin; ray 4 divergent from 2 and 3; rays 5 and 6 parallel, curved posteriad, divergent from ray 4, attaining bursal margin; ray 8 narrow, filamentous, sinuous, parallel to but not attaining bursal margin. Dorsal lobe reduced, narrow, with largely parallel margins, rounded distally, with notch; origin of dorsal lobe symmetric to slightly asymmetric to the left of midline. Dorsal ray (rays 9/10) narrow, 112–148 (134 ± 19.3) long; small opposed lateral branches with bifurcate tips at 68–70% of length from anterior, ventrally directed, papilliform; terminal bifurcation 86–91% (88 ± 2.5) of length from anterior, symmetrical or irregular, with paired lateral raylets, larger than medial raylets. Genital cone with single, triangular papilla ''0'' directed posteroventrally, situated on rounded protuberance ventral to cloaca. Genital appendages, prominent, on lateral margins of genital cone, at the level of cloaca; with multiple papilliform structures ventrally. Accessory bursal membrane dorsal to cloaca, containing distally divergent ''7'' papillae. Spicules alate, narrow, elongate, trifurcate; right spicule 351–356 (353 ± 2.9) long; left spicule 351–356 (354 ± 2.5). Main shaft of each spicule terminating in triangular cuticularized point with rounded hyaline cap; lateroventrally directed barb at 33–39 (36 ± 3.0) from proximal end of left spicule and 37–42 (39 ± 2.6) of right spicule. Dorsal process narrow, elongate, straight throughout, arising (n = 6) 47–51% (49 ± 1.4) from capitulum; extending to near the tip of the main shaft; cuticularized tip rounded, bent ventrad, contained in slightly expanded membranous extension of trabeculate ala. Ventral process long, narrow, arising at (n = 6) 36–42% (40 ± 2.4) from capitulum, consistently originating in position anterior to dorsal process; distally sinuous, curved mediad, contained in obscure membrane lacking trabeculae, terminating in angular broadened tip near level of barb. Gubernaculum, narrow, elongate (n = 2), 159–171 long, 18 wide, with poorly cuticularized head, well-defined body proximal to cloaca; 45–48% of spicule length.

Female: Body 15,850–18,860 (16,920 ± 1,683.1) long. Esophagus 1,250–1,335 (1,282 ± 46.4) long, 88–127 (109 ± 19.7) in maximum width at base; 6.7–8.4% total body length; body width at base of esophagus 176–216 (191 ± 21.8). Nerve ring 298–312 (307 ± 8), excretory pore 380–382 (380 ± 1), CP 400–410 (404 ± 5), SVGO 460–482 (468 ± 12.2), from cephalic extremity. Vulva transverse, ventral, situated 12,615–15,050 (13,488 ± 1,355.6) or 80% of body length from cephalic extremity at base of short linguiform flap. Body slightly inflated anterior to vulva; maximum body width attained at vulva, 245–367 (287 ± 69.0), decreasing immediately posterior to vulva 186–245 (207 ± 32.7). Perivulval pores situated laterally in subdorsal fields posterior to vulva, generally in interridge space, occasionally interrupting a ridge; asymmetrically located with left anterior to right; left pore approximately 325–468 (420 ± 82.5) from vulva, right pore 182–442 (312 ± 130). Genital system didelphic; ascending and descending ovaries spiral. Ovejectors (n = 1) 910 long; anterior infundibulum (n = 1) 215, sphincter + vestibule 202–220 (213 ± 10) long; posterior infundibulum (n = 1) 255, sphincter + vestibule 188–245 (218 ± 29) long. Tail conical, with rounded extremity, 325–435 (380 ± 55) long. Eggs (n = 75 in 3 specimens), 108–142 (125 ± 7.8) long, 56–75 (66 ± 4.4) wide.

Taxonomic summary

Type host: Odocoileus virginianus truei Merriam.

Type locality: Area de Conservación Guanacaste, Costa Rica (ca., 10°57′N, 85°48′W).

Specimens: Holotype male, USNPC No. 90048; allotype female, USNPC No. 90049; paratypes, 2 males and 2 females, USNPC No. 90050; collected by R. Carreno, 5 June 1999.

Etymology: Ashworthius patriciapilittae n. sp. is named in honor of Patricia Pilitt, Associate Curator of the U.S. National Parasite Collection, in recognition of her significant contributions to North American and global parasitology.

Remarks

Species of *Ashworthius* are characterized by a reduced and symmetrical dorsal lobe, with a narrow, elongate dorsal ray that bifurcates and terminates distally in 2, or more often 4, short branches; in most species, additional simple and opposed bilateral branches arise near the midlength of the dorsal ray. These contrast with species of *Haemonchus* in which the dorsal lobe typically originates in a strongly asymmetric position and contains a robust dorsal ray. The overall pattern of the cervical synlophe in species representing *Ashworthius* and *Haemonchus* is similar (Lichtenfels et al., 1994; Lichtenfels and Pilitt, 2000; Lichtenfels et al., 2002). Although there is extensive overlap among species of these genera in morphological characters, including components of the female and male genital systems, on the basis of the structure and symmetry of the dorsal ray, specimens of haemonchines in Costa Rican deer are consistent with the current concept for *Ashworthius*.

Among the 8 recognized species of *Ashworthius*, the structure of the dorsal ray for *A. patriciapilittae* n. sp., with opposed lateral branches and bifurcate tips, is unique. The trifurcate spicules and presence of a gubernaculum in males of *A. patriciapilittae* immediately distinguish it from *A. martinagliai*, *A. pattoni*, and *A. sidemi* (Le Roux, 1930; Ortlepp, 1935; Pike, 1969; Ferté and Durette-Desset, 1989); the spicule structure separates it from *A. lerouxi* (Diaouré, 1964; Pike, 1969). The narrow dorsal lobe of *A. patriciapilittae* differs from the inflat-

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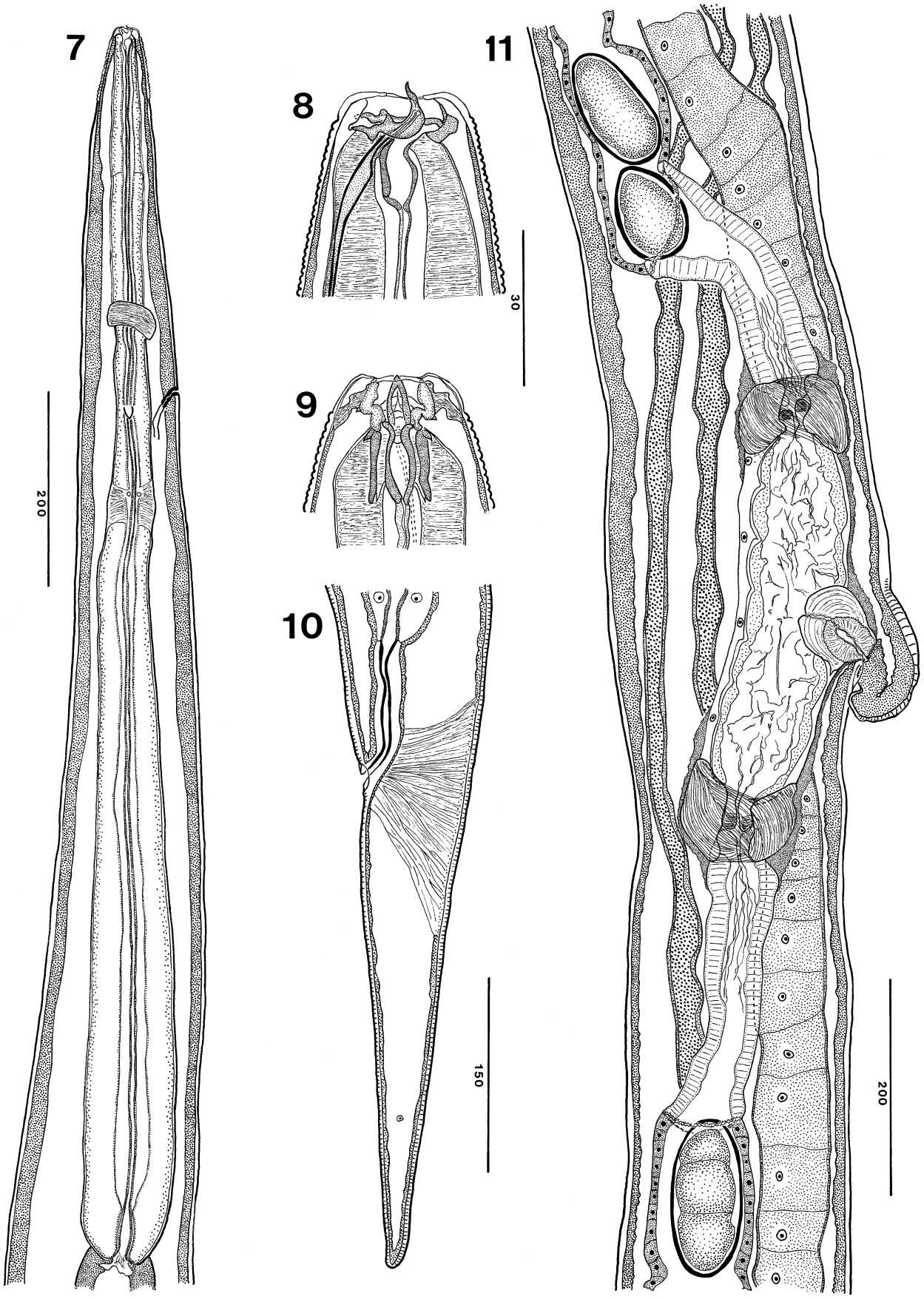
FIGURES 7–11. *Ashworthius patriciapilittae* n. sp., cervical and buccal structure and characteristics of females; drawn from the allotype; scales in micrometers. **7.** Cervical zone of female, right lateral view, showing relative positions of the nerve ring, excretory pore, cervical papillae, and subventral gland orifices. **8.** Cephalic extremity, right lateral view, showing buccal structure and prominent dorsal esophageal tooth. **9.** Cephalic extremity, ventral view, showing prominent dorsal esophageal tooth and cuticularized support structures. **10.** Tail, left lateral view. **11.** Ovejectors and vulval region, showing ventrally directed vulval flap.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ed, heart-shaped structure seen in *A. martinagliai* and *A. sidemi*, and *A. lerouxi* and *A. pattoni* lack the lateral branches on the dorsal ray, typical in *A. patriciapilittae*. In addition, specimens of *A. patriciapilittae* are substantially smaller than representatives of *A. lerouxi*, *A. pattoni*, and *A. sidemi*.

Species with strongly trifurcate spicules include *A. leporis*, *A. perrilli*, and *A. tuyenquangi* (Yen, 1961; Dróżdż, 1970; Chauhan et al., 1972). Elongate spicules with a straight and narrow dorsal process and a sinuous ventral process, a narrow dorsal lobe, and presence of a gubernaculum distinguish *A. patriciapilittae* from *A. leporis*. The latter species is characterized by spicules with short pointed processes and a broad and rounded dorsal lobe containing an irregular and sinuous dorsal ray (Yen, 1961). Spicules with a single barb, elongate dorsal and ventral processes that originate in the anterior half of the spicule, papilliform genital appendages, and a narrow dorsal lobe in *A. patriciapilittae* differ from those of *A. perrilli*. In males of the latter species, there are 2 barbs on each spicule; the processes arise from ventral and dorsal alae in the posterior portion of the spicules, and the dorsal lobe is heart-shaped and contains a dorsal ray with minuscule lateral projections (Chauhan et al., 1972).

Ashworthius patriciapilittae is morphologically most similar to *A. tuyenquangi* with regard to the trifurcate nature of the spicules, the elongate and narrow ventral and dorsal processes, and the large triangular foot (Dróżdż, 1970). Males of *A. patriciapilittae*, however, have narrower, longer spicules (351–356 μm) lacking an eyelet and dissimilar ventral and dorsal processes; the gubernaculum, 45–48% of spicule length, is also substantially larger. In *A. patriciapilittae*, the dorsal lobe is narrow with parallel margins throughout its length and contains a dorsal ray with bifurcate lateral branches that terminates distally in 4 branches. Among syntype males of *A. tuyenquangi* examined during this study, the spicules measured (n = 5) 260–305 μm, with a trifurcation at 56–57% of the total length, measured from the capitulum. The dorsal and ventral processes were similar in size, curved ventrally, and congruent distally, ending in sharp points. In contrast to the original description, a gubernaculum (n = 4), 100–132 μm in length and about 32–43% of the length of the spicules, was observed. In *A. tuyenquangi*, the dorsal lobe is inflated proximal to the genital cone and the ray ends in 2 branches.

Females of *A. patriciapilittae* are characterized by a linguiform flap at the vulva and substantially larger eggs (108–142 μm long vs. 62–64 μm long) relative to *A. tuyenquangi*. In addition, eggs of the former species are consistently larger than any described (range 62–98 μm) for the 6 other recognized species in *Ashworthius*.

A system for defining the pattern of the synlophe may be evident with respect to the position of the sublateral cords that are characteristic among species of *Haemonchus*, *Mecistocirrus* Railliet and Henry, 1912, and *Ashworthius*. In this system, ridges bounded by the sublateral cords define the lateral field and would be termed sublateral, a convention consistent with Lichtenfels and Pilitt (2000), as modified by Lichtenfels et al. (2001). Thus, in *A. patriciapilittae* and in other species with a 26-ridge system, there would be 6 lateral ridges in each field defined as 2 lateral-most and 2 pairs of sublateral; in each ventral and dorsal field there would be 7 ridges (absence of subventrals and subdorsals). In 30 ridge systems, such as *A. lerouxi* and *H. contortus* (Rudolphi, 1803), there are 8 lateral ridges in each field, where 2 are lateral-most and 3 pairs are sublaterals; there are 7 dorsal and ventral ridges. In 34-ridge systems, as in *A. sidemi*, *A. tuyenquangi*, *H. placei* (Place, 1893), and *Mecistocirrus digitatus* (Linstow, 1906), the lateral fields are identical to a 30-ridge system; the 4 additional ridges are attributed to the addition of a pair of subventral and subdorsals such that 8 ridges are present in each ventral and dorsal field. In each of these lateral systems, loss of ridges posteriad from the EIJ relates to those in the lateral fields, such that in 26-, 30- and 34-ridge systems among haemonchines, near the termination of the synlophe (either in the anterior or far posterior of the worm), the lateral field is characterized by retention of 4 ridges in each field, namely the 2 lateral-most ridges and a pair of adjacent sublaterals; there may be complete loss of the ventral and dorsal fields such as that observed in *A. patriciapilittae*.

Considerable variation was evident among the species of *Ashworthius* available for study, with regard to the number of ridges comprising the synlophe and the percentage of body length covered by the synlophe. None of the species of *Ashworthius* examined in this study had a synlophe comparable with the 26-ridge system at the EIJ characteristic for males and females of *A. patriciapilittae*. Specimens of *A. lerouxi* have 30 ridges at the EIJ, consistent with the presence of the second pair of sublaterals. Specimens of *A. sidemi* and *A. tuyenquangi* have 26 ridges at the level of the CP and increasing to 34 at the EIJ, consistent with the presence of the second pair of sublaterals and addition of paired subdorsal and subventral ridges (Lichtenfels and Pilitt, 2000).

The synlophe in males and females of *A. patriciapilittae* extends to near the caudal extremity. In males of *A. lerouxi*, the lateral synlophe extends 16–18% of the body length measured from the anterior (n = 4) and the ventral synlophe extends 12–17% (n = 2) of the body length. Examination of cross sections of 2 males and 1 female and whole mounts of *A. tuyenquangi* revealed that the synlophe in all fields terminates between 25 and 50% of the body length from the anterior. Among males and females of *A. sidemi*, the synlophe is confined to the an-

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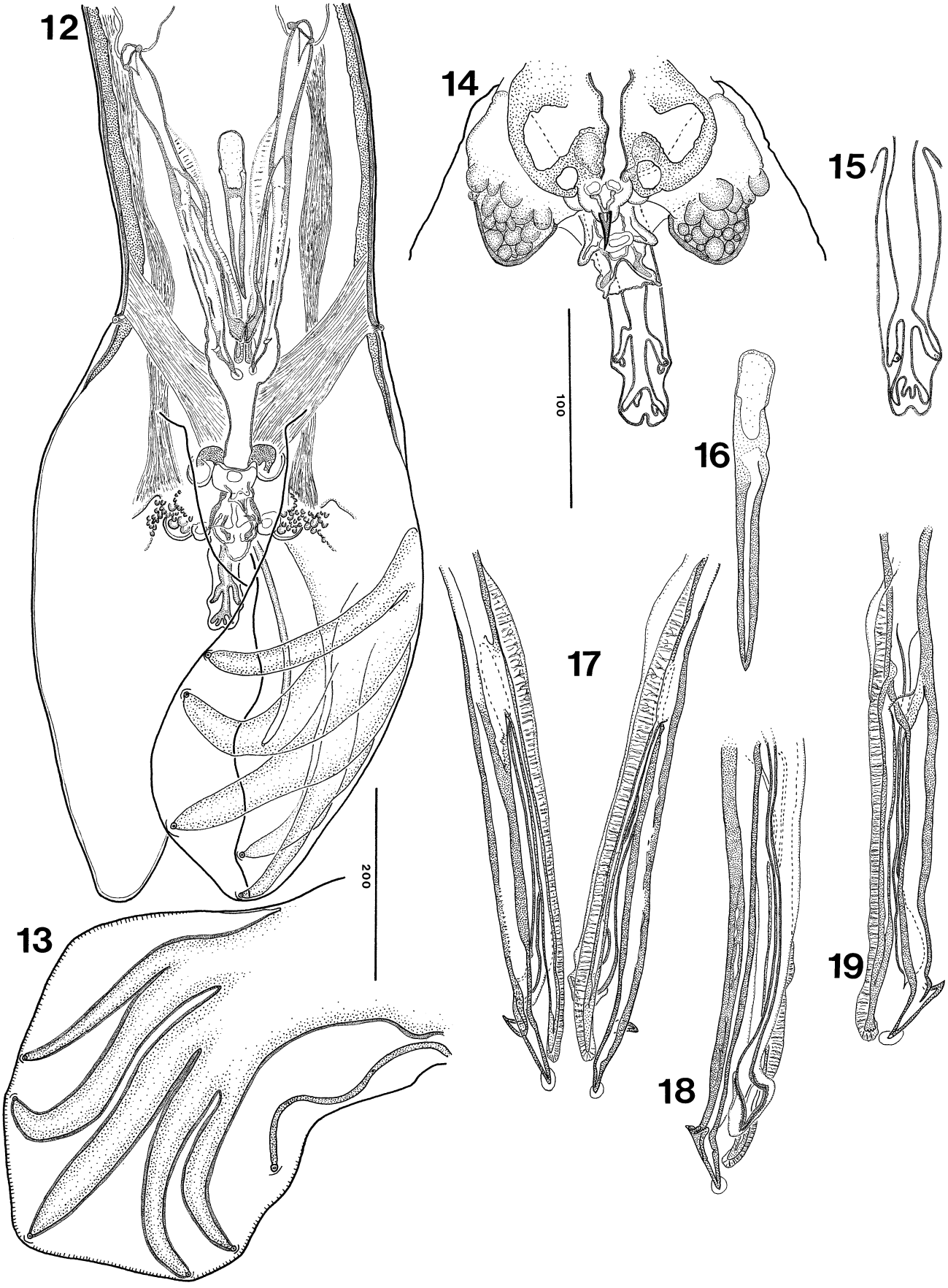
FIGURES 12–19. *Ashworthius patriciapilittae* n. sp., showing primary characteristics of males; scales in micrometers. **12.** Copulatory bursa, ventral view, showing disposition of bursal lobes, rays in left lobe, genital cone and symmetrical dorsal lobe and ray (same scale for Figs. 12, 13); drawn from paratype. **13.** Copulatory bursa, right lobe, ventral view, showing relationships of rays 2–6 and 8, with 2-1-2 pattern; drawn from paratype. **14.** Genital cone and dorsal ray, ventral view showing ventrally disposed ''0'' papilla, genital appendages bordering cloaca, paired and divergent ''7'' papillae, and symmetrical dorsal lobe and ray (same scale for Figs. 14–19); drawn from holotype. **15.** Dorsal lobe and ray, dorsal view, showing opposed lateral branches with bifurcate tips and irregular termination of dorsal ray. **16.** Gubernaculum, dorsal view. **17.** Spicule tips, dorsal view, showing termination of main shaft with lateroventrally directed barbs, narrow dorsal and ventral processes; note that origin of ventral process is consistently anterior to that of dorsal process; drawn from paratype. **18.** Spicule tip, right, ventral view; drawn from paratype. **19.** Spicule tip, right, dorsolateral view; drawn from paratype.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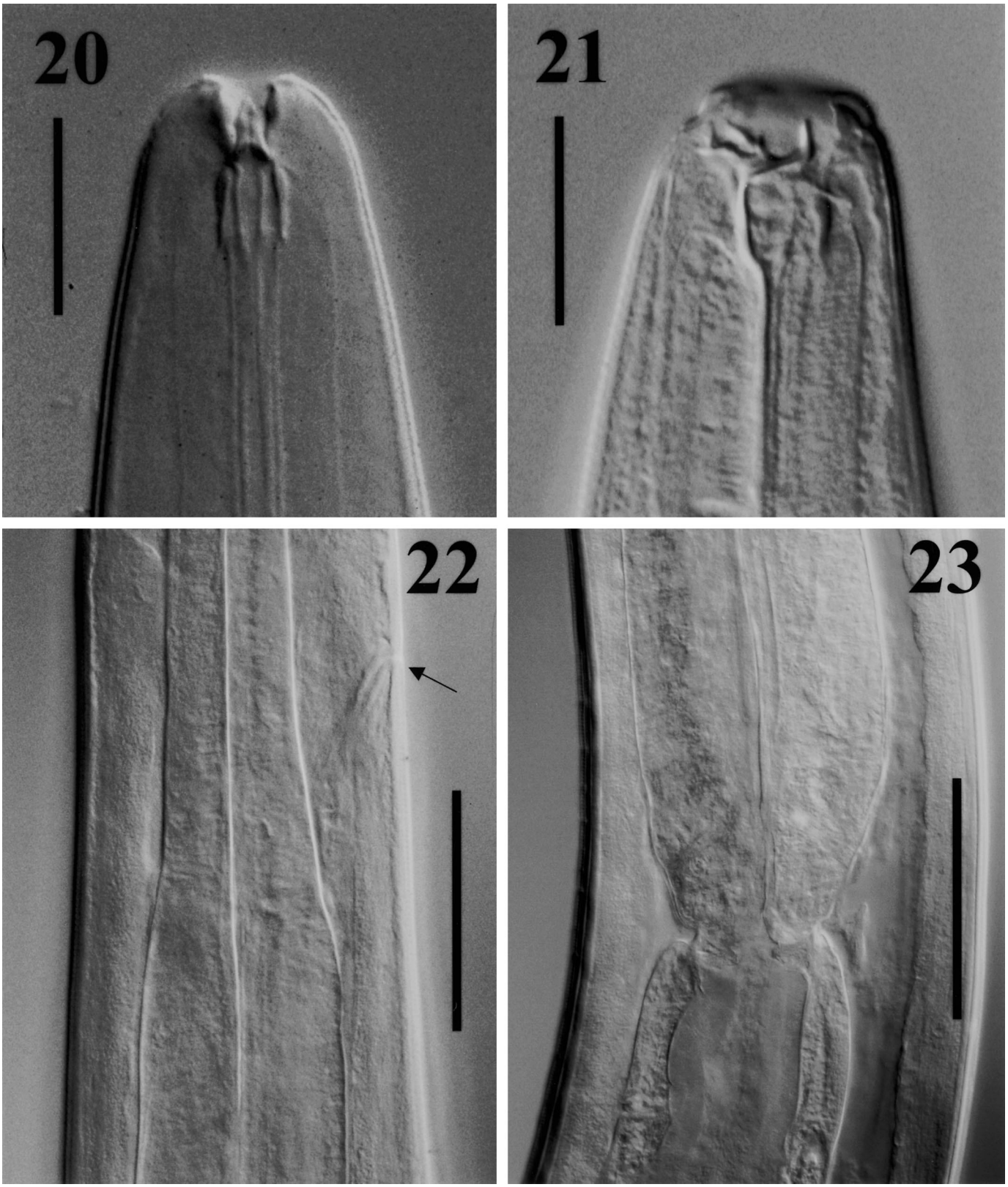
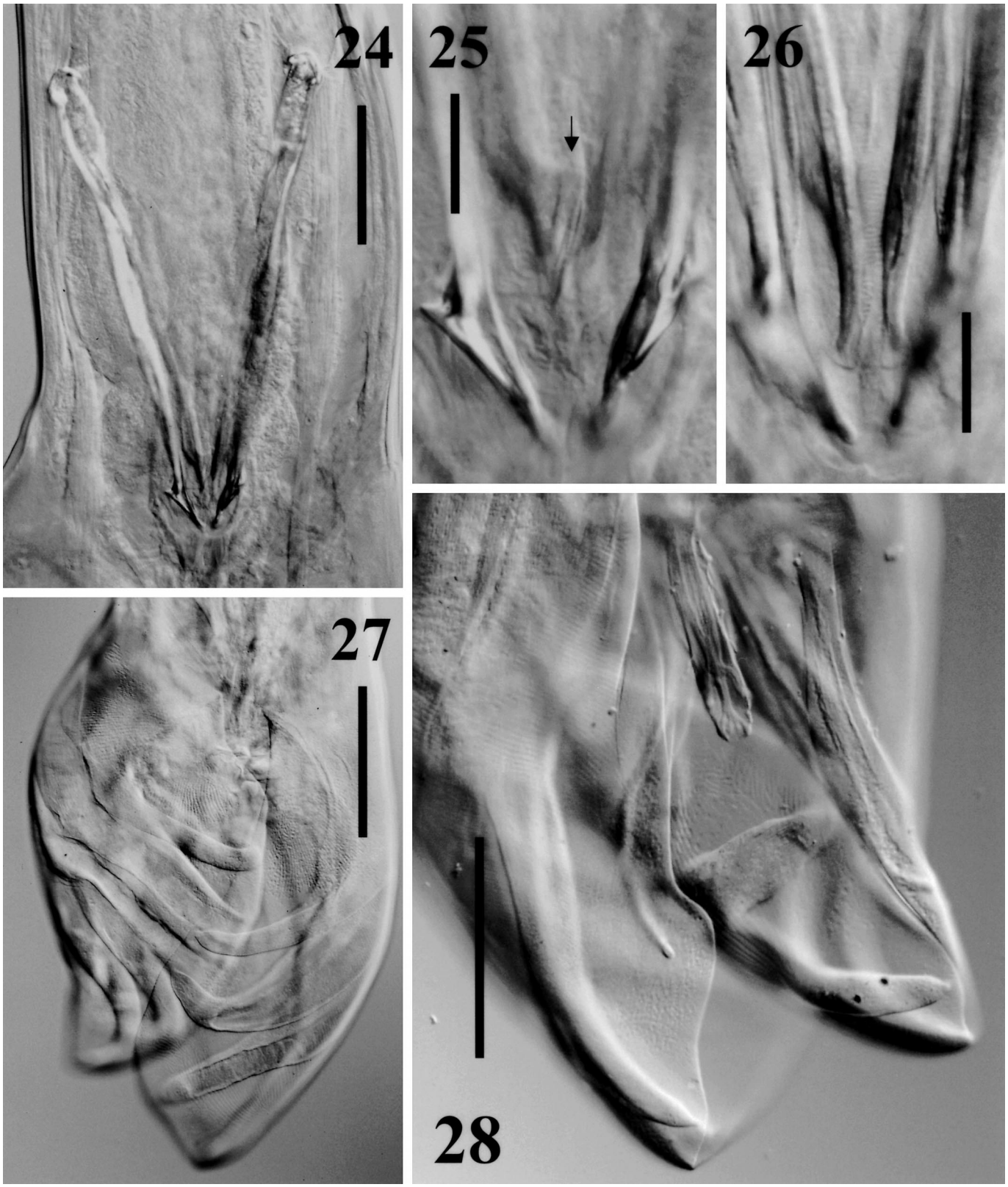
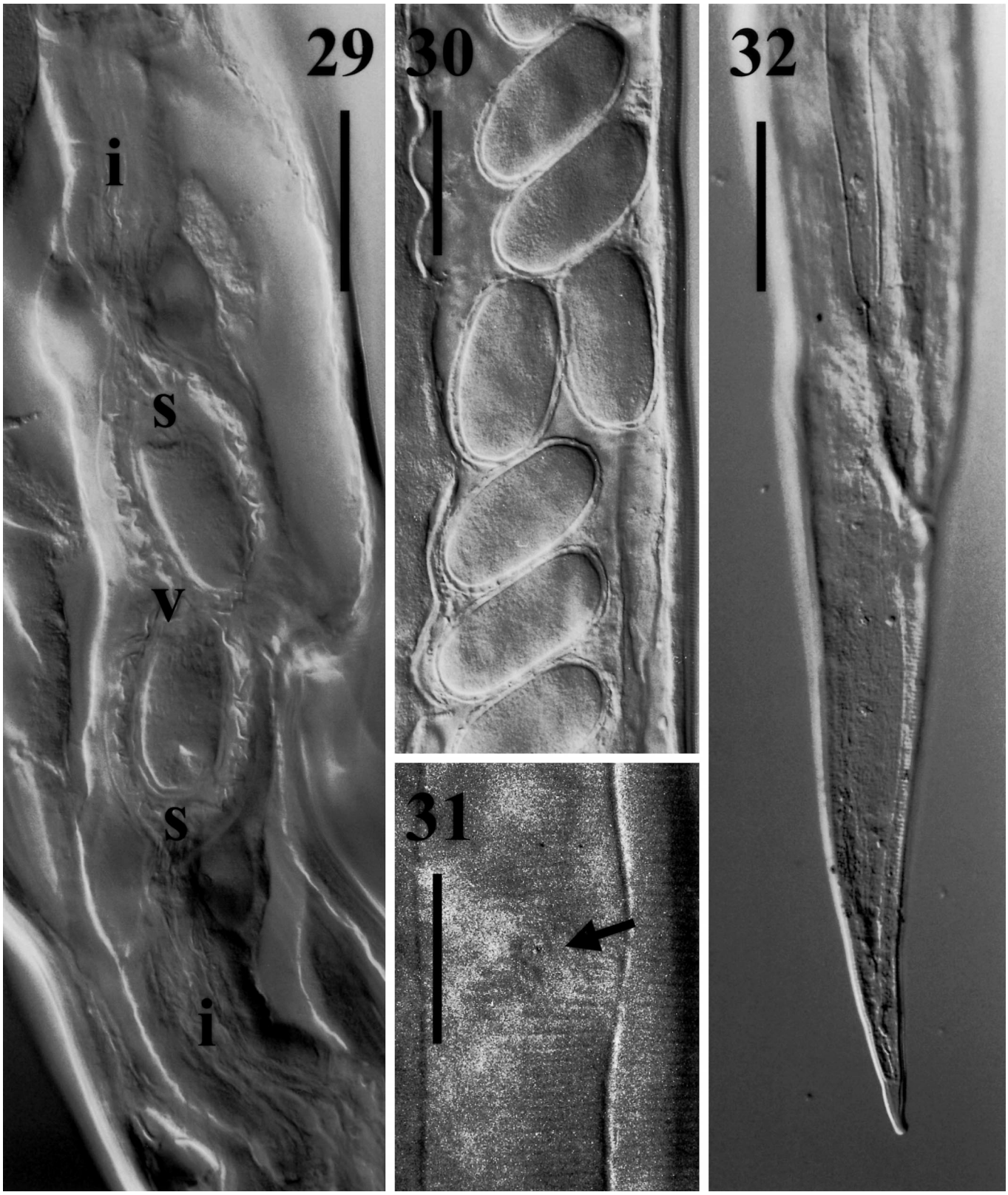


FIGURES 20–23. *Ashworthius patriciapilittae* n. sp., photomicrographs of head and esophageal region. **20.** Head of female, ventral view, showing dorsal esophageal tooth. Bar = 25 μm. **21.** Head of female, right lateral view, showing dorsal esophageal tooth. Bar = 25 μm. **22.** Cervical region of female, right lateral view, showing excretory pore (arrow) and the expansion of the esophagus posterior to the level of the SVGO. Bar = 100 μm. **23.** Esophageal–intestinal junction of male, left lateral view. Bar = 100 μm.



FIGURES 24–28. *Ashworthius patriciapilittae* n. sp., photomicrographs showing characteristics of male specimens. **24.** Spicules, ventral view, showing distal tips of main shafts, and slender dorsal and shorter ventral processes medial to main shafts slightly out of focus. Bar = 100 μm. **25.** Distal tips of spicules, ventral view, showing barbs near tips of both main shafts and the tip of the short ventral branch of the left spicule slightly out of focus (arrow). Bar = 25 μm. **26.** Distal tips of spicules, dorsal view, showing tips of dorsal branches and tips of longer main shafts. Bar = 25 μm. **27.** Copulatory bursa, ventral view, showing tips of left lateral rays. Bar = 100 μm. **28.** Copulatory bursa, dorsal view, showing narrow dorsal lobe and slender dorsal ray with small opposed lateral branches and distal branching of the dorsal ray. Bar = 100 μm.



FIGURES 29–32. *Ashworthius patriciapilittae* n. sp., photomicrographs showing characteristics of female specimens. **29.** Ovejectors, right lateral view, showing vestibule (v), anterior and posterior sphincters (s), and anterior and posterior infundibula (i). Bar = 100 μm. **30.** Eggs in uterus. Bar = 100 μm. **31.** Perivulval pore (arrow). Bar = 25 μm. **32.** Tail, right lateral view. Bar = 100 μm.

terior quarter of the body, extending in a range of about 16–24% from the cephalic extremity among specimens in *C. elaphus* from France (Ferté and Durette-Desset, 1989) or up to 24–28% among nematodes in *C. elaphus sibiricus* from Kazakhstan.

Among haemonchines in the Western Hemisphere, specimens of *A. patriciapilittae* superficially resemble and macroscopically may be confused with 3 species of *Haemonchus*, including *H. contortus*, *H. placei*, and *H. similis*, that occur in both domestic and wild ruminants (Hoberg et al., 2001). The poorly cuticularized gubernaculum, spicules with distinct dorsal and ventral processes, and symmetrical placement of the narrow dorsal ray clearly distinguish males of *A. patriciapilittae* from these species. In addition, relative to these species of *Haemonchus*, the synlophe is distinct in both males and females of *A. paticiapilittae*, where there are 26 ridges at the EIJ and the system is continuous to near the caudal extremity. This contrasts with 30 ridges in specimens of *H. contortus* and 34 in both *H. placei* and *H. similis* and a synlophe that does not extend posterior to the midbody except in the former species (Lichtenfels et al., 1994; Lichtenfels and Pilitt, 2000).

DISCUSSION

Comparative morphology for synlophes among species of *Ashworthius* and related Haemonchinae

Examination of haemonchine synlophes has revealed similarities among species in 3 genera of the subfamily, including *Mecistocirrus*, *Haemonchus*, and *Ashworthius* (Lichtenfels et al., 1994, 2002; Lichtenfels and Pilitt, 2000). Synlophes of the species in these genera may be characterized by the presence or absence of the second sublateral and subventral and subdorsal ridges, which results in a range of 26–42 ridges at the level of the EIJ. In specimens of *A. patriciapilittae*, the system of 26 ridges at the EIJ is attributable to the absence of the second sublateral ridge (a pair in each field) and paired subventral and subdorsal ridges; preliminary data suggest that this is comparable with the cervical zone in *H. dinniki* Sachs, Gibbons, and Lweno, 1973, *H. horaki* Lichtenfels, Gibbons, and Pilitt, 2001, and *H. lawrencei* Sandground, 1933 (J.R. Lichtenfels and P.A. Pilitt, pers. obs.). *Ashworthius lerouxi*, with 30 ridges at the EIJ, has a synlophe similar to that of *H. contortus* in which the second sublaterals are present. *Ashworthius tuyenquangi* and *A. sidemi*, with 34 ridges at the EIJ, have synlophes similar to *H. placei*, *H. similis*, and *M. digitatus* in which both the second sublaterals and the subventral and subdorsals are present.

The presence of similar synlophes across genera in the Haemonchinae has a parallel in the apparent uniformity of cuticular ridge systems among species in the ostertagiine genera *Spiculopteragia* Orloff, 1933, and *Mazamastrongylus* Cameron, 1935, that are otherwise diagnosed by a suite of characters specific to the genital system (Lichtenfels and Hoberg, 1993; Hoberg, 1996). The structure of the synlophe does not provide a diagnostic character that clearly distinguishes *Haemonchus*, *Ashworthius*, and *Mecistocirrus*, further indicating that the generic limits for the former 2 taxa are poorly defined.

Species of *Ashworthius* in the Western Hemisphere

Discovery of *A. patriciapilittae* n. sp. in white-tailed deer from Costa Rica represents the first report of any species of the genus in a host from the Western Hemisphere (Carreno et al., 2001). The presence of *Ashworthius* in Costa Rica can be examined in the context of competing hypotheses for cospeciation or contemporary host-switching in cervids; either *A. patriciapilittae* is a component of an endemic Central and South American fauna or it has been introduced.

Colonization of white-tailed deer in Central America by *A. patriciapilittae* could have been a consequence of translocation and introduction of domestic bovids and their parasites, coincidental with European settlement after the 1500s (Hoberg, 1997). Support for this hypothesis is minimal; however, on the basis of the apparent absence of *Ashworthius* spp. in domestic Bovinae in potential source countries including Africa and India (Pike, 1969). Cattle, *Bos taurus* Linnaeus (including zebu, *B. indicus*), and Asian water buffalo (*Bubalis bubalis* (Linnaeus)) are present on farms adjacent to the Area de Conservación (D. R. Brooks, pers. comm.), but these domestic stock are not known to be hosts for *Ashworthius* spp. In contrast to species of *Ashworthius*, another haemonchine *M. digitatus* has been disseminated globally from Asia coincidental with agricultural development and is now a dominant parasite in cattle across the Neotropical region (Lichtenfels and Pilitt, 2000).

Alternatively, the presence of *A. patriciapilittae* in Costa Rica may be indicative of an archaic distribution, reflecting an extended history with endemic cervids. Corroboration is linked to the discovery of additional species of *Ashworthius* circulating among extant species of endemic neotropical cervids including other Odocoileini (*Blastocerus* Wagner, *Mazama* (Erxleben), and *Ozotocerus* Ameghino) and Rangiferini (*Hippocamelus* Leuckart and *Pudu* Gray) from Central and South America.

Discounting a well-documented history of translocation and contemporary host-switching for *A. sidemi*, the primary distributions for *Ashworthius* spp. in cervids are limited to Eurasia and Southeast Asia. Cervinae appear to be the primary hosts for *A. sidemi* in *Cervus* spp. (Cervini) and *A. tuyenquangi* in *M. muntjak* (Muntjacini). Although these tribes are sister groups (Groves and Grubb, 1987; Amato et al., 2000; Gatesy and Arctander, 2000), the relationship of the respective *Ashworthius* spp. in cervids remains to be determined. Only *A. patriciapilittae* occurs in species of Odocoileinae, and specifically, Odocoleini, and *O. virginianus*; it is not currently known among species of Rangiferini.

There are currently insufficient data to determine if species of *Ashworthius* occur in other New World Odocoileini (extant species of *Odocoileus*, *Mazama*, *Ozotocerus*, and *Blastocerus*) or Rangiferini (extant species of *Rangifer*, *Pudu*, and *Hippocamelus*). If known species represent a coevolved assemblage, then divergence times are deep for hosts and parasites and initial diversification with cervids (Cervinae and Odocoileinae) occurred in Eurasia during the Miocene. This would be compatible with divergence within Odocoileinae in Eurasia during late Miocene, with dispersal of Odocoileini and later Rangiferini to the Nearctic, respectively, in the Miocene and Pliocene (Kurtén and Anderson, 1980; Groves and Grubb, 1987; Webb, 2000). Thus, species representing the Odocoileini and Rangiferini were already well established in North America by 3 million years before present (MYBP), and the respective tribes had independent histories of dispersal and separate evolutionary trajectories in the New World (Webb, 2000). Range extension into the neotropics and subsequent diversification for species of

both tribes occurred after 2.5 MYBP (Webb, 2000). A parallel history may be postulated for hosts and parasites. This hypothesis, however, leaves unexplained the apparent absence of *Ashworthius* in some European Cervinae and Odocoleinae (*Capreolus* Gray and *Alces* Linnaeus) and North American cervids despite the abundance of apparently suitable hosts. Resolution of the history for this assemblage is dependent on a phylogenetic context for parasites.

The biogeographic history and host associations for *Ashworthius* may parallel those among some species of Nematodirinae and Ostertagiinae in cervid hosts. *Nematodirus* Ransom, 1907, is a speciose Holarctic genus that is widespread in cervids, except 2 species, *N. odocoilei* Becklund and Walker, 1967, and *N. urichi* Cameron, 1935, occurring in *Odocoileus* spp. from North America and *Mazama americana* (Erxleben) from Trinidad, respectively (Hoberg et al., 1989; Rickard and Hoberg, 1990). In addition, species of *Mazamastrongylus* Cameron, 1935, are distributed primarily in Cervinae from Southeast Asia, Odocoileinae from the Palearctic, *Odocoileus virginianus* from the southeastern United States, and *M. americana* from Trinidad (Hoberg, 1996); a single species known from South American camelids is considered a colonizer from cervids.

Identity of *Ashworthius* spp. in the Neotropical region

The possibility also exists of previous misidentification of haemonchines, e.g., species of *Ashworthius* as *Haemonchus*, in Central and South America. Records of haemonchines in cervids are rare in these regions, although Travassos (1937) reported *H. contortus* in *Blastocerus dichotomus* Illiger and *M. americana* (listed as *M. simplicicornis*) and *H. bispinosus* (Molin, 1860) in *M. nana* Lund (listed as *C. nambi*) from South America. The latter is currently considered a synonym of *H. contortus*, according to the revision of this genus by Gibbons (1979). Cameron (1935, 1936a, 1936b) examined a limited a number of *M. americana* (listed as *M. simplicicornis*) from Trinidad but did not report any haemonchines. Díaz et al. (1977) reported abomasal nematodes and other parasites in *P. pudu* (Molina) from Chile, but haemonchines were not observed. Apparently, there is no record of parasitological survey and inventory for other species of *Mazama*, *P. mephistophiles* (De Winton), *Ozotocerus bezoarticus* (Linnaeus), *Hippocamelus antisensis* (d'Orbigny), and *H. bisulcus* (Molina).

In addition, records of *H. contortus* in *O. virginianus* across the southern United States may benefit from reexamination. This would have a bearing on developing a better understanding of the current interface of managed or agricultural and natural ecosystems, where the exchange of helminths and pathogens among wild and domestic ruminants may be facilitated at ecotones (Hoberg, 1997; Hoberg et al., 2001). Identifying endemic elements of the Neotropical and Nearctic faunas is further complicated by the potential influence of translocation of parasites (Hoberg, 1997; Hoberg et al., 2001), including those with recent introductions of European cervids such as fallow deer, *C. dama* Linnaeus, and red deer in South America (Díaz et al., 1977). Current evidence, however, indicates that only species of *Ostertagia* and *Spiculopteragia* were introduced with exotic cervids in Argentina and Chile (Díaz et al., 1977; Suarez et al., 1991).

Discovery of a species of *Ashworthius* in a cervid from the neotropics suggests that records of *Haemonchus* spp. may be in error and that renewed survey and inventory will be required to resolve the identity, host, and geographic range for haemonchines at a minimum in South and Central America. The probability that this will occur is limited by the endangered status of many populations of endemic deer (Eisenberg, 2000). Further resolution of the history for species of *Ashworthius* and other groups of gastrointestinal nematodes in the New World is dependent on the compilation of additional survey data regarding parasitism in wild and domesticated ruminants from the southern Nearctic and Central and South America (Carreno et al., 2001).

ACKNOWLEDGMENTS

We extend our sincere appreciation to Jan Dróżdż of the Stefański Institute of Parasitology, Polish Academy of Sciences, for access to specimens of *A. tuyenquangi*. We thank the scientific and technical staff of the Area de Conservación Guanacaste (ACG), Costa Rica, for support in field collections that contributed to this study. Daniel Janzen, technical advisor to the ACG, provided timely assistance in the organization and collection of specimens. Field collections by R.A.C. were further facilitated by Daniel R. Brooks, University of Toronto, who was instrumental in providing partial funding from his operating grant from the Natural Sciences and Engineering Research Council of Canada. This study was completed as a component of broader efforts for biodiversity survey and inventory of the parasites of vertebrates from the ACG, being organized and coordinated by Daniel R. Brooks.

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